121* Post-translational regulation of alginate export by an oxygen sensor in *Pseudomonas aeruginosa*

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To respond to environmental oxygen limitation, many facultative anaerobic bacteria use elaborate oxygen sensing mechanisms coupled to signalling cascades which allow optimizing a microaerobic/anaerobic life style. In *Pseudomonas aeruginosa* oxygen limitation causes up-regulation of the exopolysaccharide alginate stimulating a sessile life style. The molecular mechanisms leading to this phenotypic change are largely unknown. Here we describe a bacterial three component system (OraB/OraA/SadC) in *Pseudomonas aeruginosa* which up-regulates expression and export of the exopolysaccharide alginate under oxygen limitation via the second messenger cyclic bis-(3′-5′)-diguanylate monophosphate (c-di-GMP). During aerobic growth, cytosolic OraA blocked the activity of the membrane-bound diguanylate cyclase SadC. Anaerobic conditions relieved the inhibition of SadC by OraB, leading to c-di-GMP production and high alginate expression. Mutations in *sadC* or *oraB* in strain PAO1 resulted in a profound loss of alginate synthesis, whereas mutations in *oraA* allowed constitutive alginate synthesis under aerobic and anaerobic growth conditions. In the mucoid *P. aeruginosa* variant PDO300 carrying a *mucA* mutation, a *sadC* mutation abrogated alginate production under aerobic and anaerobic conditions, whereas overexpression of *oraA* prevented alginate production under aerobic growth. This novel post-translational regulatory mechanism links oxygen sensing with c-di-GMP-coupled signal-transduction and exopolysaccharide production in *P. aeruginosa*.

122* Molecular analysis of *Staphylococcus aureus* isolates from an ongoing prospective observational longitudinal multicenter study

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Staphylococcus aureus is not only the first but also one of the most prevalent pathogens cultured from the airways of CF-patients. The aim of this prospective longitudinal multicenter study is to dissect *S. aureus* colonization from infection by analyzing various parameters.

S. aureus isolates from primary cultures at the outpatient clinical visits were distinguished by phenotypical appearance (hemolysis, pigmentation, size), molecular typed by *spa* typing. Toxin profiles were determined for *sea, see, eta, etb, tst, seg, sej, pvl* including *agr* specificity groups by multiplex PCR.

355 *S. aureus* isolates were distinguished from 175 specimens of 100 patients from 12 different CF centers at their first visit (67 nasal swabs, 70 throat swabs, 33 sputa, 5 nasal lavages). *spa* typing revealed 83 different *spa* types which clustered in 15 different clusters. Most patients carried individual clones (59/83; 72%). Most isolates from the different sites belonged to the same clone: 44 nares and throat, 15 nares and sputa, 12 throat and sputa. The most common *spa* type, t084, appeared in specimens of 13 patients from 8 centers. The clones were positive for the following toxins: *sea* 12; seb 2; *sec* 15; *sed* 9; *eta* 4; *tst* 19; seg 64; *seh* 10; *sei* 67; *sej* 6;. PVL 1; hlg 113; *agr*I 59, *agr*II 30, *agr*III 26, *agr*IV 4.

The majority of clones appeared only in single patients. Only few clones were distributed between several patients in different centers. Many isolates were positive for the pyrogenic superantigens with *sei* being the most detected gene indicating the virulence potential of *S. aureus* in the background of CF.

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123 Prevalence of MRSA in patients with cystic fibrosis (CF) in R. Macedonia

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Aim: Increasing prevalence of MRSA in CF population is an emerging problem. This study is aimed to determine the rate of MRSA in our CF patients in previous 3 years.

Methods: The study included 90 (1–29 y) CF patients who regularly attended our CF center. We retrospectively analyzed all microbiological results for CF patients from 2007 to 2009 focusing on detection of MRSA.

Results: We identified 16 patients (mean age 15±8 y) with MRSA (17.8%) in 2009, 6 (6.6%) in 2008 and 3 (3.33%) in 2007 retrospectively. Multidrug resistant MRSA was detected in 10 patients (9%). One child died from MRSA sepsis (8 y) and one has bacterial endocarditis (16 y). Eradication therapy was preformed to all patients using combined dual IV antibiotic treatment accompanied by hygienic directives over 3 weeks. This was followed by a 4 week period with oral antibiotic therapy.

Conclusion: Prevalence of MRSA in our CF patients in the last year was very high in comparison with previous years. We suspect that the reason is transmission between the patients, because we have limited space and we have no opportunity to separate our patients.

124 Comparison and characterization of MRSA isolates from paediatric CF patients

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Introduction: Although MRSA is increasingly cultured from the respiratory tract of CF patients, its contribution to declining lung function remains unclear. This study aimed to characterize MRSA isolated from CF patients at two paediatric centres using molecular typing methods. Levels of delta-haemolysin (d-hly), the translation product of the major effector molecule of the *agr* system, were also determined.

Methods: Isolates were characterized by pulsed-field gel-electrophoresis (PFGE), SCC*mec* typing (multiplex PCR), *agr* typing (PCR-RFLP) and *spa* tpying. Levels of d-hly produced by isolates grown under both anaerobic and aerobic conditions were compared by measuring *in vitro* haemolysis assays.

Results: PFGE determined that isolates from each centre formed distinct clusters with *spa* types similar to those in circulation in local hospital populations. D-hly levels were greater under anaerobic vs aerobic conditions for 19/28 isolates (67.9%), with no significant difference apparent for 8/28 (28.1%) isolates. Initial results suggest that this was most marked among isolates with *agr II*.

Conclusions: MRSA cultured from CF patients appear to reflect strains circulating in the local hospital background, as suggested by SCC*mec* and *spa* typing, and additional work is therefore required to investigate routes of transmission or exposure to MRSA in the CF clinic. The implications of increased production of d-hly under anaerobic conditions are under further investigation, as this may result in the down-stream modulation of other virulence factors, which may potentially play a key role in MRSA infection in CF.